**Procedure:** CLIA-Waived

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| Review Date | Revision Date | Signature |
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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21CFR809.10). Prepared in accordance with the guidelines recommended by the National Committee for Clinical Laboratory Standards, Villanova, PA 19085; NCCLS Document GP2-A2.

# QUICKVUE+ Infectious Mononucleosis test

**CLIA Waived Procedure for Whole Blood**

WHOLE BLOOD PROCEDURE WAIVED CLIA '88

## INTENDED USE

QuickVue+ Infectious Mononucleosis test is a rapid Color ImmunoChromatographic Assay (CICA®) for the detection of Infectious Mononucleosis IgM heterophile antibodies in whole blood. This test is intended for use as an aid in the diagnosis of Infectious Mononucleosis. For use by healthcare professionals.

## SUMMARY AND EXPLANATION

Infectious Mononucleosis (IM) is usually a self-limiting disease that is caused by the Epstein-Barr Virus (EBV).[1,2] The most common symptoms are fatigue, pharyngitis, fever, lymphadenopathy, splenomegaly and hepatitis.[3] In rare cases, complications may develop including severe thrombocytopenia, hemolytic anemia, pericarditis, myocarditis, pneumonitis, Reye's syndrome, encephalitis and other neurological syndromes. In industrialized countries, the peak incidence of IM occurs between 14 and 18 years of age. In developing or densely populated countries, most children become infected before 3 years of age and symptoms may be mild or clinically inapparent.[4,5]

During the acute phase of illness, certain heterophile antibodies appear in 85–90% of IM cases. These antibodies, known as IM heterophile antibodies, are primarily of the IgM class.[6,7] Although the exact mechanism leading to IM heterophile antibody expression has not been determined, the antibodies are specifically associated with the disease. The IM heterophile antibodies are usually demonstrable one week after the onset of illness, peak at two to four weeks, and decline to low levels by 12 weeks.[2] Heterophile antibodies have been detected in patients' serum over one year after the onset of illness.[8]

Reliable laboratory diagnosis of IM has been performed for over fifty years based on the detection of IM heterophile antibodies. These heterophile antibodies are directed against antigens found in bovine, sheep and horse erythrocytes. QuickVue+ Infectious Mononucleosis test utilizes an extract of bovine erythrocytes which gives a greater sensitivity and specificity than similar extracts prepared from sheep and horse erythrocytes.[9,10] The Forssman antibody, which can interfere with some IM heterophile antibody assays[7], does not interfere with the QuickVue+ Infectious Mononucleosis test.

## PRINCIPLES

The QuickVue+ Infectious Mononucleosis test assay uses Color ImmunoChromatographic Assay (CICA) technology for the qualitative detection of human heterophile IM antibodies (IgM class) in whole blood.

The Reaction Unit consists of a plastic housing containing a membrane strip which provides the solid support for the immunochromatographic assay. The right end of the membrane provides contact with the sample well. The sample well contains an absorbent pad which provides an even flow of the sample fluid (from right to left ) along the membrane. The first zone of the membrane (which is covered by the Reaction Unit label) is coated with blue latex beads that are conjugated to goat anti-human IgM antibodies (antibody-blue latex). Two agents are immobilized on the second zone of the membrane, which is exposed in the "Read Result" window. These agents include blue latex beads (non-conjugated) immobilized on the membrane to provide a pre-printed blue horizontal line. The second agent is a bovine erythrocyte extract, which is immobilized on the vertical line. The third zone of the membrane (exposed in the "Test Complete" window) contains an agent capable of binding the antibody-blue latex to provide the vertical "Test Complete" sign. An absorbent pad is situated at the left end of the membrane to retain fluid after the reaction is completed. A drying agent is enclosed in the Reaction Unit to stabilize the reactive agents.

In the Test Procedure, whole blood is added to the "Add" well, followed by the addition of the Developer. As the sample/developer fluid moves by capillary action across the first zone of the membrane, it mobilizes the antibody-blue latex. The fluid continues to move the antibody-blue latex across the membrane to the immobilized bovine erythrocyte extract (antigen) zone. If the specific IM heterophile antibodies are present in the sample, a "sandwich" of solid phase/IM antibody/antibody-blue latex is formed. The vertical line will appear resulting in a positive sign (+) visible in the "Read Result" window, which indicates the presence of IM heterophile antibody. If the antibody is not detected, the "Read Result" window will only contain the pre-printed blue horizontal line, indicating a negative (–) result. As the fluid continues to move the antibody-blue latex across the membrane, it comes in contact with the reagent in the "Test Complete" window. A blue line will appear, indicating the test is complete.

## REAGENTS AND MATERIALS PROVIDED

Each QuickVue+ test kit contains enough reagents and materials for 20 tests.

1. Reaction Unit (20)
   Test strip contains anti-human IgM, immobolized extracted bovine erythrocyte antigens and sheep antibody.
2. Developer (5 mL)
   Detergent, 0.2% sodium azide.
3. Mono Negative Control (1 mL)
   Normal human serum diluted in saline solution, 0.2% sodium azide.
4. Mono Positive Control (1 mL)
   IgM heterophile antibody positive human plasma diluted in saline solution, 0.2% sodium azide.
5. Sample Pipettes (20)
6. Capillary Tubes (20).
7. Package Insert (1)
8. Procedure Card (1)

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Vacutainer tubes: EDTA or heparin for venipuncture whole blood procedure.
2. Finger Lancet for fingertip blood procedure.

## STORAGE

Store kit at room temperature 59–86°F (15–30°C), out of direct sunlight. Do not freeze.

## PRECAUTIONS

1. For *in vitro* diagnostic use.
2. DO NOT use materials after expiration date. DO NOT mix components from different lots or different kits.
3. DO NOT interchange caps among reagents.
4. All patient samples and controls should be handled as if they were capable of transmitting disease. Observe established precautions against microbiological hazard throughout all procedures and follow the standard procedures for proper disposal of samples.

5. Developer contains sodium azide. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Copious quantities of water should be used to flush Developer down a sink.
6. **Warning: Potential Biohazardous Material**
   Each donor unit of human serum or plasma used in the preparation of the Positive and Negative Controls was tested by an FDA-approved method for the presence of the antibody to human immunodeficiency virus type HIV-1/HIV-2, as well as hepatitis B surface antigen (HBsAg) and anti-HCV, and found to be negative. Nevertheless, caution should be used in handling and disposing of these items at biosafety level 2, as recommended in the Centers for Disease Control/National Institutes of Health Manual, Biosafety in Microbiological and Biomedical Laboratories, 1984.

## SAMPLE COLLECTION AND STORAGE

1. Collect an anticoagulated blood sample following standard laboratory procedures.
2. Whole blood containing either EDTA or heparin as an anti-coagulant may be used immediately without centrifugation; or may be stored at 2–8°C for up to 72 hours.
3. Fingertip blood should be tested immediately after the sample is taken.
4. Whole blood samples in which cell lysis has occurred will cause a red background to appear in the "Read Result" window. However, the result remains valid.

***Recommendations for Capillary Whole Blood Fingerstick Sampling:***

- Wash patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- Massage the hand without touching puncture site by rubbing down the hand and finger towards the tip.
- The side of the middle or ring finger is the preferred puncture site.
- Puncture the skin with the lancet. Wipe away first sign of blood.
- Gently rub the hand from wrist to palm to finger in order to form a rounded drop of blood over the puncture site. Avoid squeezing around the puncture site.
- Touch the capillary tube to the blood until filled.

## QUALITY CONTROL

### External Quality Control

External Positive and Negative Controls are provided in the kit for quality control. Positive and Negative Controls should be tested with each new lot or shipment of test materials and as otherwise required by your laboratory's standard Quality Control procedures.

1. **External Positive Control:** Put one drop of Positive Control in the "Add'" well. Process the control as you would a patient sample. A positive signal is indicated by a vertical blue line in the "Read Result" window, resulting in a positive sign (+).
2. **External Negative Control:** Put one drop of Negative Control in the "Add" well. Process the control as you would a patient sample. A negative signal is indicated by a horizontal blue line (–), in the "Read Result" window.

### Internal Procedural Control

1. **Internal Positive Procedural Control:** A blue line in the "Test Complete" window is considered an internal positive procedural control. If the test has been performed correctly and the Reaction Unit is working properly, this indicator will appear.
2. **Internal Negative Procedural Control:** A clear background in the "Read Result" window is considered an internal negative procedural control. If the test has been performed correctly and the Reaction Unit is working properly, the background will clear to give a discernible result.

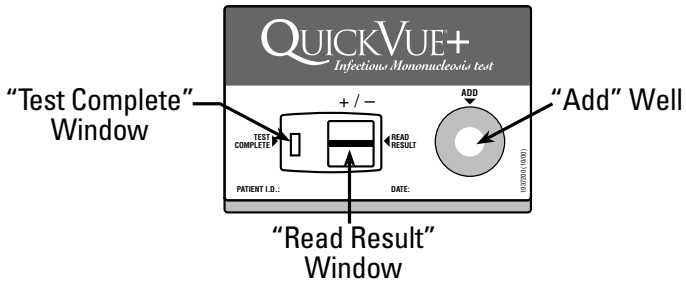
### Procedural Notes

1. DO NOT open the foil pouch until you are ready to perform the test.
2. Several tests may be run at one time.
3. To avoid cross-contamination, use a new disposable Sample Pipette for each sample.
4. To avoid contamination, do not touch the tip of the Developer bottle to the Reaction Unit.
5. Commercial controls other than Quidel's should not be used with QuickVue+ Infectious Mononucleosis test because they may contain additives which will interfere with the test performance.

## TEST PROCEDURE – WHOLE BLOOD

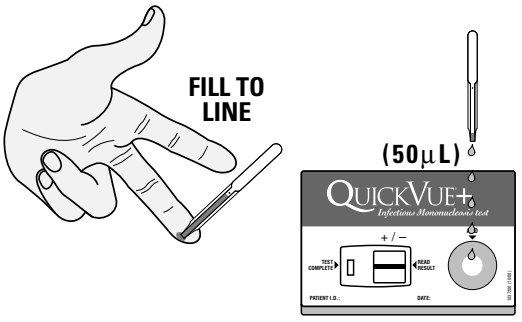
Read all of the procedural instructions before running patient samples.

Remove the Reaction Unit from the pouch and place it on a well lit and level surface.
**The "Read Result" window contains a horizontal blue line pre-printed on the membrane.**




### Capillary Tube Procedure

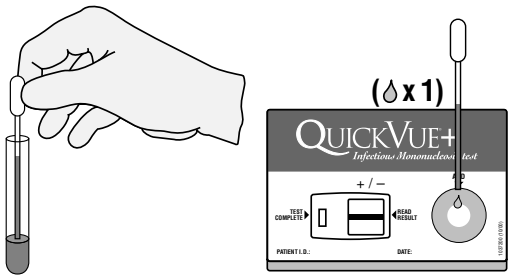
For fingertip blood, fill the capillary tube (50 µL) to line.
**Dispense all blood into the "Add" well.**



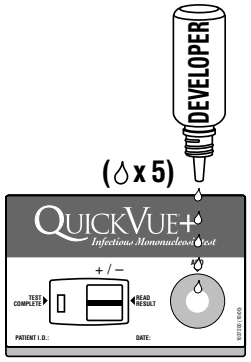

OR

### Venipuncture Procedure

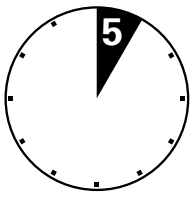
For whole blood samples in tubes, use the sample pipette provided.
**Place one drop of sample in the "Add" well.**




Hold the Developer bottle vertically.
**Add 5 drops of Developer to the "Add" well.**




**Read results at 5 minutes.**



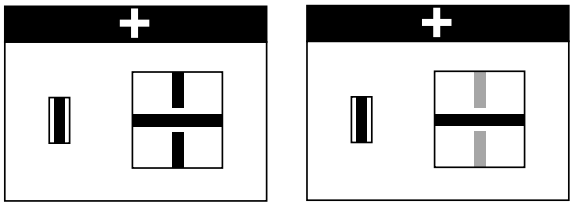

***"Test Complete" line must be visible by 10 minutes.***

# INTERPRETATION OF RESULTS

***FOR PATIENT SAMPLES, POSITIVE AND NEGATIVE CONTROLS***

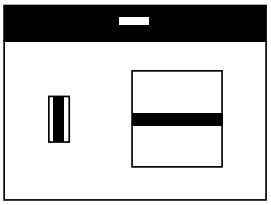
## Positive Result

**Any shade** of a blue vertical line forming a (+) sign in the "Read Result" window along with the blue "Test Complete" line, is a positive result. **Even a faint blue vertical line should be reported as a positive.**




## Negative Result

No blue vertical line in the "Read Result" window along with the blue "Test Complete" line, is a negative result.

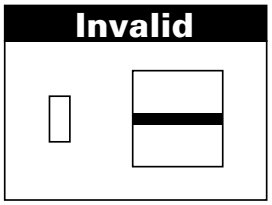



## Invalid Result

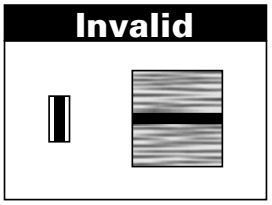
Test results are invalid:

- If after 10 minutes no signal is observed in the "Test Complete" window. (View #1.)
- If after 10 minutes a blue color fills the "Read Result" window. (View #2.)

View #1




View #2




An invalid result indicates either the test was not performed correctly or the reagents are not working properly.

Should an invalid result occur, re-test the sample using a new Reaction Unit.

If the problem continues, contact Technical Support toll-free in the U.S. at (800) 874-1517. Outside the USA, contact your local representative.

## LIMITATIONS

1. As is the case of any other diagnostic procedure, the results obtained by this kit yield data that must be used in addition to other information available to the physician.
2. QuickVue+ Infectious Mononucleosis test is a qualitative test for the detection of IM heterophile antibodies.
3. A negative result may be obtained from patients at the onset of the disease due to antibody concentration below the sensitivity of this test kit. If symptoms persist or increase in intensity, the test should be repeated.
4. Some segments of the population who contract Infectious Mononucleosis do not produce measurable levels of heterophile antibodies. Approximately 50% of children under 4 years of age who have IM may test as IM heterophile antibody negative.[4]

## PERFORMANCE CHARACTERISTICS

QuickVue+ Infectious Mononucleosis test was compared to two other commercially available test kits: an EIA, and a slide hemagglutination test. The results obtained using QuickVue+ Infectious Mononucleosis test were substantially equivalent to the results obtained using these other tests and are summarized below.

**Table 1:** In this study, a total of 511 serum, plasma and whole blood samples were tested using QuickVue+ Infectious Mononucleosis test and a commercially available EIA.

| | EIA Positive | EIA Negative |
|---|---|---|
| **QuickVue+ IM** Positive | 74 | 1 |
| **QuickVue+ IM** Negative | 0 | 436 |
| **TOTAL** | 74 | 437 |

Of the 511 samples, 74 were found to be positive by EIA and also positive by the QuickVue+ Infectious Mononucleosis test; similarly, 437 were found to be negative by EIA and 436 were also negative by the QuickVue+ Infectious Mononucleosis test.
Based on this data, **specificity was 99.8%** (436/437), and **sensitivity was >99.9%** (74/74). **Overall agreement was 99.8%** (510/511).

**Table 2:** In this study, a total of 511 serum, plasma and whole blood samples were tested using QuickVue+ Infectious Mononucleosis test and a commercially available slide hemagglutination test.

| | Slide Positive | Slide Negative |
|---|---|---|
| **QuickVue+ IM** Positive | 74 | 1 |
| **QuickVue+ IM** Negative | 1 | 435 |
| **TOTAL** | 75 | 436 |

Of the 511 samples, 75 were found to be positive by the slide hemagglutination test and 74 were also positive by the QuickVue+ Infectious Mononucleosis test; similarly, 436 were found to be negative by the slide hemagglutination test and 435 were also negative by the QuickVue+ Infectious Mononucleosis test.

Based on this data, **specificity was 99.8%** (435/436), and **sensitivity was 98.7%** (74/75). **Overall agreement was 99.6%** (509/511).

## ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel's Technical Support Number 800-874-1517 (toll-free) or 858-552-1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time. If outside the United States contact your local Quidel office or distributor.

## REFERENCES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## LOG SHEET

QUICKVUE+ Infectious Mononucleosis test

**Lot Number:** _____

*Record Built-in Procedural Controls on the first patient tested each day*

**Exp. Date:** _____

| | Date | Patient Name | Extraction Reagent Control | Positive Procedural Control (C=Blue Line) | Test Results at 5 minutes | Technician |
|---|---|---|---|---|---|---|
| **1** | | | | | | |
| **2** | | | | | | |
| **3** | | | | | | |
| **4** | | | | | | |
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